

ing of PEG-bis-thiols, poly(N-isopropylacrylamide)-bis-thiols (NIPAAm-bis-thiols), poly(acrylic acid)-bis-thiols, poly(methacrylic acid)-bis-thiols, poly(styrene sulfonate)-bis-thiols, poly(amide)-bis-thiols, and any combination thereof.

7. The 3D printed hydrogel according to claim 6, wherein the bis-thiol containing cross-linking agent is a PEG-bis-thiol.

8. The 3D printed hydrogel according claim 5, wherein the biopolymers are selected from the group consisting of thiol-gelatin, thiol-cellulose, thiol-chitosan, thiol-hyaluronic acid, and any combination thereof.

9. The 3D printed hydrogel according to claim 5, wherein the small molecule is dithiothreitol (DTT).

10. The 3D printed hydrogel according to claim 5, wherein the bioactive molecule is selected from the group consisting of short chain peptides with at least two cysteine amino acid groups, inert short chain peptides, enzyme responsive short chain peptides, matrix metalloproteinase (MMP) responsive peptides, and any combination thereof.

11. The 3D printed hydrogel according to claim 10, wherein the bioactive molecule is an MMP-responsive bis-cysteine peptide.

12. The 3D printed hydrogel according to claim 11, wherein the MMP-responsive peptide is selected from the group consisting of GCIPVSLRSGCG, GCRDGPQGIWGQDRCG, GCRDPLGLDRCG, GCRDEAPLKQDRCG, and any combination thereof.

13. The 3D printed hydrogel according to claim 1, wherein the maleimide containing polymer is PEG-maleimide and the bis-thiol containing cross-linking agent is selected from the group consisting of PEG-bis-thiol, an MMP-responsive peptide, and a combination thereof.

14. The 3D printed hydrogel according to claim 1, wherein the maleimide containing polymer is PEG-maleimide substituted with at least one bioactive molecule selected from RGD, YIGSR and IKVAV and the bis-thiol containing cross-linking agent is selected from PEG-bis-thiol, an MMP-responsive peptide, or a combination thereof.

15. The 3D printed hydrogel according to claim 13 or 14, wherein the MMP-responsive peptide is GCIPVSLRSGCG, GCRDPLGLDRCG, or a combination thereof.

16. The 3D printed hydrogel according to claim 1, wherein the maleimide containing polymer is gelatin maleimide and the bis-thiol containing cross-linking agent is PEG-bis-thiol.

17. The 3D printed hydrogel according to any one of claims 1 to 16, wherein the molar ratio of maleimide containing polymer to bis-thiol containing cross-linking agent is in the range of 10:1 to 1:10.

18. The 3D printed hydrogel according to any one of claims 1 to 17 containing cells.

19. The 3D printed hydrogel according to claim 18, wherein the cells are suspended within a part of the hydrogel or the cells are substantially uniformly suspended throughout the hydrogel.

20. The 3D printed hydrogel according to claim 18 or 19, wherein the concentration of printed cells is in the range of 1×10^5 to 5×10^8 cells/mL.

21. The 3D printed hydrogel according to any one of claims 18 to 20, wherein the cells are selected from the group consisting of liver cells, gastrointestinal cells, pan-

creatic cells, kidney cells, lung cells, tracheal cells, vascular cells, skeletal muscle cells, cardiac cells, skin cells, smooth muscle cells, connective tissue cells, corneal cells, genitourinary cells, breast cells, reproductive cells, endothelial cells, epithelial cells, fibroblast, neural cells, Schwann cells, adipose cells, bone cells, bone marrow cells, cartilage cells, pericytes, mesothelial cells, cells derived from endocrine tissue, stromal cells, stem cells, progenitor cells, lymph cells, blood cells, endoderm-derived cells, ectoderm-derived cells, and mesoderm-derived cells, and any combination thereof.

22. The 3D printed hydrogel according to any one of claims 1 to 21 further comprising a bioactive molecule.

23. The 3D printed hydrogel according to claim 22, wherein the bioactive molecule is a free bioactive molecule or bound to the maleimide containing polymer, the bis-thiol containing cross-linking agent, or both the maleimide containing polymer and the bis-thiol containing cross-linking agent.

24. The 3D printed hydrogel according to claim 22 or 23, wherein the bioactive molecule is selected from the group consisting of a peptide, MMP-responsive peptide, protein, polysaccharide, drug, therapeutic agent, antibody, small molecule inhibitor, kinase inhibitor, phosphatase inhibitor, antigen, pathogen, platelet, growth factor, cytokine, amino acid, nutrient, conditioned media, antibiotic, antiviral, RNA, and any combination thereof.

25. The 3D printed hydrogel according to claim 24, wherein the bioactive molecule is collagen.

26. The 3D printed hydrogel according to any one of claims 1 to 24, further including a cell culture medium.

27. A method of preparing a 3D printed hydrogel, the method comprising the steps of:

providing a polymer bio-ink comprising a maleimide containing polymer;

providing an activator comprising a bis-thiol containing cross-linking agent having at least two thiol functional groups; and

printing the polymer bio-ink and the activator to form a 3D printed hydrogel.

28. The method according to claim 27, further comprising providing cells to form a 3D printed hydrogel containing cells.

29. The method according to claim 28, wherein the cells are present in the polymer bio-ink, in the activator, both in the polymer bio-ink and the activator, or in a separate medium prior to 3D printing.

30. The method according to any one of claims 27 to 29, wherein the 3D printed hydrogel is formed within 30 minutes or less, or 10 minutes or less, or 1 minute or less, or 30 seconds or less, or 10 seconds or less, or 1 second or less, from the printing of the polymer bio-ink and the activator.

31. The method according to any one of claims 27 to 30, wherein the polymer bio-ink, the activator, or both are adjusted to about pH 7.4 prior to printing.

32. The method according to claim 31, wherein the pH is adjusted using NaOH.

33. A cell assay comprising a 3D printed hydrogel containing cells according to any one of claims 18 to 21.

34. Use of a 3D printed hydrogel containing cells according to any one of claims 18 to 21 for a cell assay.

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